

**ODESSA NATIONAL MEDICAL UNIVERSITY**  
**Department of General and Clinical Pharmacology and Pharmacognosy**

**METHODOLOGICAL DEVELOPMENT**

**Course: "Pharmacognosy"**

**practical lesson for students on the topic:**

"Methods of pharmacognosy: microscopic analysis. Analysis of crushed LRS of different morphological groups, microchemical reactions to some classes of BAS. "

Course: 3rd Faculty: medico-pharmaceutical

**Approved on methodical  
meeting of the department  
"30" August 2024  
Protocol № 1  
Head department  
MD, prof. JV Rozhkovsky**



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**Odessa - 2024**

**1. Topic:** "Methods of pharmacognosy: microscopic analysis. Analysis of crushed LRS of different morphological groups, microchemical reactions to some classes of BAS. " - 4 years

## **2. Relevance of the topic.**

One of the main tasks of practical pharmacognosy is to determine the authenticity (identity) of medicinal plant raw materials. Both macroscopic and microscopic methods of analysis play an important role in performing this task. Histochemical reactions to various classes of natural compounds contained in plant tissues also help to establish reliability to a great extent.

Knowledge and skills to determine the reliability of medicinal plant raw materials will be used by pharmacists in their practical activities in the process of procurement of raw materials, its acceptance from the public or analysis.

## **3. Objectives of the lesson:**

*3.1 General objectives:* to master the methods of microscopic and histochemical analysis of medicinal plant raw materials.

*3.2 Educational goals:* formation of a professionally significant substructure of personality with relevant aspects of deontological, ecological, legal, psychological, patriotic, professional responsibility.

*3.3 Specific objectives:*

**- to know** (level of assimilation according to Bezpalk - II):

1. What is the purpose of microscopic analysis;
2. Features of the technique of preparation of temporary drugs;
3. Indifferent and brightening liquids;
4. Reagents for mucus, essential oil, tannins and anthracene derivatives;
5. Conductive tissues of plants;
6. Mechanical fabrics;
7. Primary and secondary root structure.

Based on theoretical knowledge of the topic and practical work:

- **Master the techniques (be able)** (level of assimilation according to Bezpalk - III):
- to establish the identity of the crushed raw material by anatomical features;
- to detect the localization of BAS in medicinal plant raw materials.

#### 4. Interdisciplinary integration

№ p.p.	discipline	know	be able
1	2	3	4
1.	Previous disciplines: 1. Botany  2. Organic chemistry  3. Analytical chemistry	Characteristic features of the families of the studied plants. Morphology of stem, bark, leaves, flower, fruit, root and rhizome. Anatomical structure of the leaf, bark, fruit, root, rhizome.  Physical and chemical properties of polysaccharides, glycosides, terpenoids, aromatic derivatives, heterocycles.  Methods of acid-base titration (neutralization) and permanganometry	Use a microscope, prepare surface preparations and cross-sections.  Carry out qualitative reactions; purification of organic compounds.  Work with analytical scales, measuring vessels, photoelectro-colorimeter, use methods of chromatography on paper and in a thin layer of sorbent.
2.	The following disciplines: 1. Physical and colloid chemistry	Solubility of solids and liquids in liquids. Distillation. Raoul's law. Konovalov's law. Vapor pressure and composition over mutually insoluble liquids. Buffer solutions. Polarography. Potentiometric titration. Adsorption. Ion exchange adsorption. Chromatography: paper, column, in a thin layer of sorbent, gel chromatography.	

	<p>2. Pharmacy technology of drugs</p> <p>3. Industrial technology of medicines</p> <p>4. Clinical pharmacology</p> <p>5. Pharmaceutical chemistry</p> <p>6. Organization and economics of pharmacy</p>	<p>Methods of measuring mass and volume. Preparation of powders or liquid drugs for internal and external use. Analysis of prepared liquid drugs using a burette system.</p> <p>Conditions of industrial preparation of medicines. Principles of organization of pharmaceutical production of various dosage forms: liquid, solid, soft, injectable solutions, etc. Machines, devices, equipment for the production of medicines.</p> <p>Pharmacodynamics and pharmacokinetics of drugs. The pattern of action of drugs on the human body and its corresponding reactions. Basic principles of treatment in terms of drug selection, evaluation of their effectiveness and safety.</p> <p>Methods of qualitative and quantitative study of drugs.</p> <p>Pharmaceutical service management. Storage of medicines. Control and analytical service, organization of its work. Accounting for inventory and cash. Economic analysis of the pharmacy.</p> <p>Management and entrepreneurship. Organization as an object of management. Connecting processes in management. Human Resource Management Pharmaceutical Marketing Management. Pharmaceutical market research. International marketing.</p>	
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	7. Management and marketing in pharmacy		
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### 5. Content of the topic (text and thesis), graphological structure of the lesson.



### 6. Plan and organizational structure of the lesson.

№№ р.р.	The main stages of the lesson, their functions and content.	Learning objectives in the levels of mastery.	Means of training and control.	Materials on methodical forensuring the visibility of the lesson, control the knowledge of those who teachis.	Term (in minutes or in%) of the total class time.
1	2	3	4	5	6
1	Preparatory stage Organization of classes Setting learning goals Homework check	II	Oral interview on the topic	Methodical works for students, album	1% 2% 25%
2	The main stage Conducting practical lesson	III	Herbariums of medicinal plants, LRS, reagents		50%
3	The final stage				

	Testing and assessment of practical skills	II- III	Herbariums of medicinal plants, LRS, reagents	Methodical works for students, album	5%
	Checking the final level of knowledge	II- III		Tests and situational tasks	15%
	Providing homework with a reference to the literature				3%

## 7. Materials on methodological support of the lesson

### 7.1. Control materials for the preparatory stage of the lesson: questions, tasks, tests.

#### Tests.

1. Plant mucus is a polysaccharide of various compositions. What reaction, based on the physical properties of mucus, is used to detect them:

- A. Reaction with methylene blue
- B. Reaction with Sudan
- C. Deposition reaction
- D. Reaction with safranin
- E. Reaction with aniline sulfate

2. Which method is most suitable for microscopic analysis of medicinal raw materials, consisting of coarse woody underground organs:

- A. Maceration
- B. Boiling
- C. Distillation with water
- D. Cold softening
- E. Steam softening

3. As a result of the reaction with chlorine-zinc-iodine under a microscope observed blue-violet or purple color of cell membranes. Determine the type of histochemical reaction:

- A. Reaction to pure fiber
- B. Reaction to sugar
- C. Reaction to fats
- D. Reaction to carbohydrates
- E. Reaction to mucus

4. To determine the reliability of medicinal raw materials used the reaction using 5% sodium hydroxide solution or ammonium hydroxide. Observed red or purple-red color, which indicates the presence of:

- A. anthracene derivatives
- B. Tannins
- S. Flavonoids
- D. polysaccharides
- E. saponins

5. To identify medicinal raw materials, its slice is placed in a drop of Lugol's solution. Observe the cherry color, which indicates the presence in the raw material:

- A. Starch
- V. Fats
- S. Mucus
- D. Carbohydrates
- E. Pure fiber

7. Specify the histochemical reaction, as a result of which a slice of raw material is placed for several hours in a solution of Sudan 111, then washed with 50% alcohol and transferred to glycerol. Observe the orange-red color:

- A. Reaction to fats

- B. Reaction to essential oils
- C. Reaction to tannins
- D. Reaction to starch
- E. Reaction to mucus

8. To establish the authenticity of the raw material, to its broth was added a few drops of ferric chloride or 1% aqueous solution of ferric ammonium alum. A black and blue color is formed, which indicates the presence of raw materials:

- A. Tannins
- B. Anthracene derivatives
- S. Saponins
- D. Alkaloids
- E. Polysaccharides

9. During the histochemical reaction, a slice of medicinal raw material was placed for a few minutes in a solution of Sudan-3, and then reviewed in water or glycerin. Obtained a green color, which indicates the presence of raw materials:

- A. Essential oils
- V. Fats
- S. Starch
- D. Slime
- E. Tannins

10. When conducting microscopic analysis of marshmallow roots, it is necessary to determine the presence of starch grains in plant cells. Which reagent can be used to do this:

- A. Lugol's solution
- B. Ammonium hydroxide
- C. Concentrated sulfuric acid
- D. Alcohol solution of naphthol

## E. Thymol solution

### **Question:**

1. What is the purpose of microscopic analysis.
2. Describe the technique of preparation of permanent and temporary drugs.
3. How to make a cross section of the bark, root ..
4. How to make a cross section of small seeds
4. Name the inclusive liquids.
5. Name the brightening liquids
6. Name reagents for mucus, starch, fiber, woody elements, fatty and essential oils.
7. Name the formula of calcium oxalate inclusions.
8. How do vessels differ in the nature of the secondary wall thickening.
9. Name the different types of hair, glands, the shape of the epidermis.
10. What reactions determine the presence of inulin and starch in LRS.
11. Elements of conductive mechanical fabric.
12. Pharmacognostic definition of "Bark".
13. Anatomical structure of the root and rhizome.
14. Anatomical structure of the cortex
15. Microdiagnostic signs of the cortex and underground organs, their differences.

### **7.2. Materials of methodical support of the main stage of employment: professional algorithms, orientation maps for formation of practical abilities and skills, educational tasks.**

#### **The list of educational practical tasks that must be performed during the practical laboratory lesson:**

**Task 1.** To study the anatomical diagnostic signs of the cortex.

1. To study the general nature of the anatomical structure of the cortex on a permanent cross-sectional preparation.
2. Draw a diagram of the structure of the cortex:

- Cortical layer;
- Collenchyma;
- Primary bark (indicate the location of mechanical elements);
- Secondary cortex (indicate the width and shape of the core rays and the location of mechanical elements).

3. Prepare a micropreparation of bark powder. To study at low and high magnification the mechanical elements and crystalline inclusions of calcium oxalate.

At high magnification, draw and mark:

- Bast fibers with crystal-bearing coating;
- Stony cells.

**Task 2.** To study the anatomical diagnostic signs of roots and rhizomes

1. Set the type of structure of the root and rhizome on permanent cross-sectional preparations.

2. Draw a diagram of the anatomical structure of each object.

3. Identify the types of vascular bundles.

4. To study at high magnification the elements of xylem and phloem.

5. To study the nature of the structure:

- Integumentary tissue;
- Primary and secondary cortex;
- Core rays;
- Calcium oxalate crystals;
- Cells with mucus and essential oil.

6. To study at low and high magnification the types of vessels on the longitudinal section of the root.

7. Draw and mark them.

**Task 3.** Carry out histochemical reactions to detect BAR in LRS.

*Reaction to mucus* - with a cross section of marshmallow root.

*Reaction to essential oil* - with a cross section of the rhizome of calamus

*Reaction to anthracene derivatives* - with a cross section of buckthorn bark.

*Reaction to tannins* - with a cross section of oak bark and viburnum bark

Record the observations in a laboratory journal.

### **Histochemical reactions.**

#### **1. Reaction to mucus**

- *With methylene blue.*

The slice is placed for a few minutes in a solution of methylene blue in alcohol (1: 5000), then transferred to glycerol; the mucus turns blue.

- *with copper sulfate and alkali.*

The slice is placed for 5-10 minutes in a saturated solution of copper sulfate, washed with water and transferred to a 50% solution of potassium hydroxide; mucus turns blue (plants of the mallow family), or green (plants of the lily family).

#### **2. Reaction to essential oils**

The slice is placed for a few minutes in a solution of Sudan 3, and then viewed in water or glycerin. The essential oil turns green. To distinguish essential oils from fats use a solution of methylene blue in water (0.1 gmethylene blue in 500 ml of water). The objects are placed in the reagent for a few minutes and then viewed in water or glycerin. The essential oil turns blue.

#### **3. Reaction to anthracene derivatives**

The slice is placed on a glass slide in a drop of 5% solution of sodium hydroxide or ammonium hydroxide, add a drop of glycerin, cover with a cover glass and observe under a microscope red or purple-red color of tissues in which anthracene derivatives are localized.

#### **4. Reaction to tannins ..**

The section is placed in a drop of ferric chloride or 1% aqueous solution of ammonium alum, cover with a cover glass and observe the color of the drug under a microscope. Fabrics containing tannins are dyed black-blue or black-green.

#### **5. Reaction to fats**

The slice is placed for several hours in a solution of Sudan 3, then washed with 50% alcohol and transferred to glycerol. Sudan 3 colors fats in orange-red color.

#### **6. Reaction to starch.**

Apply a drop of Lugol's solution to the slice, cover with a cover glass and observe under a microscope. Starch grains acquire a blue or purple color.

### **7. Reaction to woody fiber.**

The slice is placed on a glass slide in a 1% solution of floroglucin in alcohol, the reagent is aspirated with filter paper, a drop of concentrated hydrochloric acid is applied to the slice and after 1-2 minutes a drop of glycerin is added; cover with a cover glass and study under a microscope at low magnification. The woody membranes of the cell acquire a cherry color.

### **Instructional materials for mastering professional skills, abilities:**

Methods of work performance, stages of performance:

- a) get the necessary LRS
- b) to study and describe the appearance of the obtained LRS, to draw LRS
- c) to conduct LRS training
- d) to study the anatomical and diagnostic features of roots and rhizomes
- e) to study the anatomical and diagnostic features of fruits and leaves
- f) record the observations in a laboratory journal

### **7.3. Control materials for the final stage of the lesson: tasks, tasks, tests, etc.**

#### **Tests:**

1. Alkaloids from medicinal plant raw materials are isolated by extraction in the form of bases. Specify which solvent is used for this:

- A. Chloroform
- B. Acidified alcohol
- C. Acetic acid
- D. Acidified water
- E. Sodium hydroxide solution

2. Determine the reagent, which is the only solvent in which the fiber swells and then dissolves:

- A. Schweizer's reagent
- B. Dragendorff's reagent
- C. Mayer's reagent
- E. Wagner's reagent
- E. Wiesseling reagent

3. To establish the authenticity of marshmallow root, the State Pharmacopoeia requires histochemical reactions to determine:

- A. Mucus
- V. Starch
- S. Routine
- D. Alizarin
- E. Tanin

4. A reaction with floroglucin and hydrochloric acid was performed. The result was a cherry color. Specify the histochemical reaction:

- A. Reaction to woody fiber
- B. Reaction to alkaloids
- C. Reaction to fats
- D. Reaction to mucus
- E. Reaction to resins

5. To detect which biologically active substance of plant origin, the reaction with methylene blue is used:

- A. Mucus
- V. Zhirov
- S. Small
- D. Starch

E. Fiber

6. Specify the biologically active substance contained in the plant material, which gives a positive qualitative reaction to Lugol's solution:

A. Starch

B. Fiber

S. Jury

D. Resins

E. Slime

7. A batch of marshmallow root was delivered to the warehouse. To confirm the authenticity of the slice applied a drop of ammonia solution, a yellow color was obtained, indicating the presence in the raw material:

A. Mucus

B. Tannins

S. Comedy

D. Pectic substances

E. Vitamin C

## **8. Literature**

### **Basic literature**

1. Фармакогнозія: підручник (I—III р. а.) / І.А. Бобкова, Л.В. Варлахова. – 3-є видання Всеукраїнське спеціалізоване видавництво «Медицина» 2018, 504с.
2. Фармакогнозія: базовий підручн. для студ. вищ. фармац. навч. закл.(фармац. ф-тів) IV рівня акредитації / В.С. Кисличенко, І.О. Журавель, С.М. Марчишин та ін.; за ред. В.С. Кисличенко. – Харків: НФаУ: Золоті сторінки, 2015. - 736 с.
3. Навчальний посібник з дисципліни «Фармакогнозія» / Я. В. Рожковський, Б. В. Приступа, І. А. Бойко, Н. В. Герасимюк, В. В. Черногорюк -: Методична розробка кафедри фармакогнозії ОНМедУ. – Одеса: ОНМедУ, 2019 – 51 с.
4. Державна Фармакопея України: в 3 т. / Державне підприємство «Український

науковий фармакопейний центр якості лікарських засобів». – 2-е вид. – Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2015. – Т. 1. – 1500 с.


**Additional literature:**

1 Державна Фармакопея України: в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. – Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2014. – Т. 3. – 732 с.

2. Практикум з ідентифікації лікарської рослинної сировини: навч. посіб. / [В. М. Ковальов, С. М. Марчишин, О. П. Хворост та ін.] ; за ред. В. М. Ковальова, С. М. Марчишин. – Тернопіль: ТДМУ, 2014. – 250 с.

**10. The topic of the next lesson:**

“Carbohydrates. Glycosides. Chemical analysis of LRS. LR and LRS, which contain polysaccharides: species of marshmallow, plantain, coltsfoot (mother-and-stepmother), flax, kelp. Determination of the swelling index of raw materials. ”.

*Methodical recommendations were made by*  *associate professor Boyko IA*

*(Signature)*